Application No.: 10/616,082
Amendment Date: 22-Oct-08
Reply to Office Action of: 14 May 2008

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claim 1 (Currently amended): A method for producing a recombinant glycoprotein in a uni- or multicellular fungal host cell which includes an α-1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity and is diminished or depleted in the activity of an initiating α-1,6-mannosyltransferase and which produces N-glycans comprising GlcNAcMan5GlcNAc2 structures, the method comprising the step of expressing in the host cell a nucleic acid encoding a chimeric mannosidase enzyme comprising

(a) a *D. melanogaster* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Gls1-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, P.Sec-m, Mnn9-s, Van1-s, Van1-m, Van1-l, Anp1-s, Anp1-m, Anp1-l, Hoc1-s, Hoc1-m, Hoc1-l, Mnn10-m, Mnn11-s, Mnt1-m, J3-m, Ktr1-s, Ktr2-s, Gnt1-s, Gnt1-m, Gnt1-l, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-m, Mnn1-s, Mnn1-m, Mnn1-l, Mnn6-s, and Mnn6-m or

(b) a *C. elegans* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Gls1-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, Van1-s, Van1-m, Van1-l, Anp1-s, Hoc1-m, Mnn10-s, Mnn10-m, Mnn10-l, Mnn11-s, Mnn11-m, Mnt1-s, Mnt1-m, Mnt1-l, D2-s, D2-m, D9-m, J3-m, Ktr2-s, Gnt1-s, Gnt1-m, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-s, Mnn5-m, Mnn1-s, Mnn1-m, and Mnn6-m,

wherein said chimeric enzyme in (a) or (b) is capable of hydrolyzing *in vivo* more than 40-50 percent of the Man α-1,3 and/or Man α-1,6 linkages of a GlcNAcMan5GlcNAc2 substrate ~~that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a Manα1,3 and Manα1,6 glycosidic linkage to the extent that at least 10% of the Manα1,3 and/or Manα1,6 linkages of the substrate are hydrolyzed *in vivo*~~,

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whereby expression of said chimeric mannosidase produces one or more desired N-glycan structures on a recombinant glycoprotein expressed in said host cell wherein the desired N-glycan is characterized as having at least the oligosaccharide branch Manα1,3 (Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn.

Claim 2 (Currently amended :A method for producing a recombinant glycoprotein in a uni- or multicellular fungal host cell which includes an α-1,2-mannosidase and a GlcNAc transferase I (GnT I) and is diminished or depleted in the activity of an initiating α-1,6-mannosyltransferase and which produces N-glycans comprising $GlcNAcMan_5GlcNAc_2$ structures, the method comprising the step of expressing in the host cell a nucleic acid encoding a chimeric mannosidase enzyme comprising

(a) a *D. melanogaster* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Gls1-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, P.Sec-m, Mnn9-s, Van1-s, Van1-m, Van1-l, Anp1-s, Anp1-m, Anp1-l, Hoc1-s, Hoc1-m, Hoc1-l, Mnn10-m, Mnn11-s, Mnt1-m, J3-m, Ktr1-s, Ktr2-s, Gnt1-s, Gnt1-m, Gnt1-l, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-m, Mnn1-s, Mnn1-m, Mnn1-l, Mnn6-s, and Mnn6-m or

(b) a *C. elegans* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Gls1-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, Van1-s, Van1-m, Van1-l, Anp1-s, Hoc1-m, Mnn10-s, Mnn10-m, Mnn10-l, Mnn11-s, Mnn11-m, Mnt1-s, Mnt1-m, Mnt1-l, D2-s, D2-m, D9-m, J3-m, Ktr2-s, Gnt1-s, Gnt1-m, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-s, Mnn5-m, Mnn1-s, Mnn1-m, and Mnn6-m,

wherein said chimeric enzyme in (a) and (b) is capable of hydrolyzing *in vivo* more than 40-50 percent of the Man α-1,3 and/or Man α-1,6 linkages of a $GlcNAcMan_5GlcNAc_2$ substrate ~~that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a Manα1,3 and Manα1,6 glycosidic linkage~~, whereby expression of said chimeric mannosidase produces one or more desired N-glycan structures on a recombinant glycoprotein expressed in said host cell, wherein the desired N-glycan is produced within the host cell at a yield of at least 10 mole percent and wherein the desired N-glycan is characterized as having at least the oligosaccharide branch Manα1,3 (Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn.

Claims 3-5 (Cancelled)

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Claim 6 (Original): The method of claim 1 or 2, wherein the oligosaccharide substrate is characterized as Manα1,3 (Manα1,6 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; Manα1,3 (Manα1,3 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; GlcNAcβ1,2 Manα1,3 (Manα1,6 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; GlcNAcβ1,2 Manα1,3 (Manα1,3 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; Manα1,3 (Manα1,3 Manα1,6 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; GlcNAcβ1,2 Manα1,3 (Manα1,3 Manα1,6 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; Manα1,2 Manα1,3 (Manα1,3 Manα1,6 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; Manα1,2 Manα1,3 (Manα1,3 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; Manα1,2 Manα1,3 (Manα1,6 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn or high mannan.

Claim 7-9 (Cancelled)

Claim 10 (Currently amended): The method of claim 1 or 2, wherein ~~the mannosidase enzyme~~ the chimeric mannosidase enzyme comprises a Class IIx mannosidase ~~activity~~ catalytic domain fused to a cellular targeting signal peptide that targets the chimeric enzyme to the secretory pathway of the host cell.

Claim 11 (Previously presented) : The method of claim 10, wherein the Class IIx mannosidase enzyme has a substrate specificity for Manα1,3 (Manα1,6 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; Manα1,3 (Manα1,3 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; or Manα1,2 Manα1,3 (Manα1,3 Manα1,6 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn.

Claim 12 (Currently amended): The method of claim 1 or 2, wherein ~~the mannosidase enzyme~~ the chimeric mannosidase enzyme comprises a Class III mannosidase ~~activity~~ catalytic domain fused to a cellular targeting signal peptide that targets the chimeric enzyme to the secretory pathway of the host cell.

Claim 13 (Previously presented) : The method of claim 12, wherein the Class III mannosidase enzyme has a substrate specificity for (Manα1,6 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; (Manα1,3 Manα1,6) Manβ1,4-GlcNAc β1,4-GlcNAc-Asn; or high mannans.

Claim 14 (Previously presented) : The method of claim 1 or 2, wherein the mannosidase enzyme is overexpressed.

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Claim 15 (Previously presented) : The method of claim 1 or 2, wherein the mannosidase enzyme is further capable of hydrolyzing a Manα1,2 linkage.

Claim 16 (Previously presented) : The method of claim 1 or 2, wherein the mannosidase enzyme has a pH optimum of from about 5.0 to about 8.0.

Claim 17 (Canceled)

Claim 18 (Previously presented) : The method of claim 1 or 2, wherein the mannosidase enzyme is localized within the secretory pathway of the host cell.

Claim 19 (Previously presented) : The method of claim 1 or 2, wherein the mannosidase enzyme is localized within at least one of the ER, Golgi apparatus or the trans Golgi network of the host cell.

Claims 20-25 (Cancelled)

Claim 26 (Original): The method of claim 1 or 2, further comprising the step of isolating the glycoprotein from the host cell.

Claim 27 (Original) : The method of claim 1 or 2, wherein the host cell is selected from the group consisting of *Pichia pastoris, Pichia finlandica, Pichia trehalophila, Pichia koclamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia guercuum, Pichia pijperi, Pichia stiptis, Pichia methanolica, Pichia sp., Saccharomyces cerevisiae, Saccharomyces sp., Hansenula polymorpha, Kluyveromyces sp., Kluyveromyces lactis, Candida albicans, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Trichoderma reesei, Chrysosporium lucknowense, Fusarium sp., Fusarium gramineum, Fusarium venenatum* and *Neurospora crassa.*

Claim 28 (Original) : The method of claim 27, wherein the host cell is *Pichia pastoris.*

Claim 29 (Original) : The method of claim 1 or 2, wherein the glycoprotein is a therapeutic protein.

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Claim 30 (Original) : The method of claim 29, wherein the therapeutic protein is selected from the group consisting of erythropoietin, cytokines, coagulation factors, soluble IgE receptor α-chain, IgG, IgG fragments, IgM, interleukins, urokinase, chymase, urea trypsin inhibitor, IGF-binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein, angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin, α-1-antitrypsin and α - feto protein.

Claims 31 – 56 (Cancelled)

Claim 57 (Previously presented) : The method of claim 1, wherein the desired N-glycan comprises an oligosaccharide structure selected from the group consisting of $Man_3GlcNAc_2$, $GlcNAcMan_3GlcNAc_2$, and $Man_4GlcNAc_2$.

Claim 58 (Previously presented): The method of claim 2, wherein the desired N-glycan comprises an oligosaccharide structure selected from the group consisting of $Man_3GlcNAc_2$, $GlcNAcMan_3GlcNAc_2$, and $Man_4GlcNAc_2$.

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